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Hussain SA
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Wani SA
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Ahmad SR
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Sofi AH
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Rather MA
FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Ahmad SM
FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Gani A
Department of FST, University of Kashmir, Srinagar, Jammu and Kashmir, India

Farooq S
FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Nazir T
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Corresponding Author:
Hussain SA
Division of Livestock Products Technology, FVSc & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Jammu and Kashmir, India

Oregano essential oil as a natural alternative to augment the aerobic refrigerated shelf life of meat emulsion, studies thereof

Hussain SA, Wani SA, Ahmad SR, Sofi AH, Rather MA, Ahmad SM, Gani A, Farooq S and Nazir T

Abstract
The present study was conducted to evaluate the antioxidative and antimicrobial potential of Oregano Essential Oil (OEO) as a potential alternative to synthetic preservative, butylated hydroxy toluene (BHT) in complex food system i.e., meat emulsion, stored aerobically at refrigeration temperature. The emulsion was divided into four treatments *viz*., Control (without any additive), Positive control (BHT-200ppm), O100 (OEO-100 ppm) and O200 (OEO-200 ppm). All the treatments were stored in low density polyethylene (LDPE) packs and subjected to refrigerated storage (4±1 ºC) for a period of 1 week. The antioxidative (TBARS, PV, DPPH-RSA), antimicrobial (TPC) and sensory (Overall acceptability) evaluation of all the emulsion treatments was conducted on 0, 3rd, 5th and 7th day of storage. On the basis of these parameters it was concluded that both OEO-200 ppm and BHT-200 ppm enhanced the shelf life of meat emulsion by 2-3 days and OEO-200 ppm displayed better activity than OEO-100 ppm. Thus, OEO could be used as a natural alternative to replace BHT, a synthetic preservative to augment the shelf life of meat emulsion.

Keywords: Meat emulsion, natural antioxidants, oregano essential oil, Butylated hydroxyl toluene (BHT), refrigerated storage, quality

Introduction
Meat and meat products are excellent sources of high-quality proteins, essential amino acids, B-vitamins, minerals, and various other micronutrients (Marangoni *et al*., 2015) [1]. The presence of particular nutrients in the meat offer a number of health benefits like prevention of age-related muscle loss (essential amino acids), promotion of gut health (nucleotides and nucleosides), and reduction of blood pressure due to inhibition of angiotensin I converting enzyme (bioactive peptides) (Ramachandraiah *et al*., 2018) [2].

The modern life style has created a huge demand for convenience food items including both ready-to-eat as well as ready-to-cook food products which is mainly related to their rapid and easy cooking (Ferreira *et al*., 2017) [3]. Meat emulsion being an intermediate ready to cook meat product, if available ready-made as a convenience food item would enable quick product development.

However, the oxidative and microbial degradative processes are more pronounced in ground and emulsion based meat products owing to increased surface area for the action of molecular oxygen and other intrinsic pro-oxidants. The addition of fat for stable emulsion formation further aids in this oxidative degradation as does the physical disruption of muscle and cell integrity which diminishes the endogenous protective antioxidant systems (Bekhit *et al*., 2013) [4]. These processes cause sensory degradation of the product leading to consumer rejection. In addition, there is decreased shelf life, nutritional loss as well as formation of toxic substances that may pose risks to human health. (Domínguez *et al*, 2019; Cao *et al*., 2018) [5, 6]. Furthermore, thermal processing of the meat accelerates the development of oxidation by rapid generation of free radicals and destroys the endogenous antioxidant systems making cooked meat more susceptible to oxidative degradation than the raw meat (Xiong *et al*., 2020; Serpen *et al*., 2012) [7, 8]. Thus, all the processes utilized in preparation of emulsion based meat products like size reduction, grinding, cooking and reheating after cold storage promote the oxidative degradation of the meat products (Domínguez *et al*, 2019) [5].

As such, it would be in the best interests of society and the global meat industry if raw emulsion could be preserved as such and marketed as a ready-to-cook (RTC) convenience meat product, suitably packaged in appropriate packages with cooking instructions.

Traditionally several synthetic preservatives like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tert-butyl-hydroquinone (TBHQ) have been used in the food industry to prevent deteriorative changes. However owing to their negative impact on human health their usage is discouraged. In the past decade, there has been a major shift in the consumer preferences. Today's intelligent consumer demands safe, wholesome and minimally processed food which is free from harmful pathogens and synthetic preservatives (Jayasena and Jo 2013) [9]. Keeping this in view, the industry has shifted its trend towards the use of natural preservatives and it is well documented that lipid oxidation and microbial spoilage can be efficiently controlled or minimized by using natural antioxidants and antimicrobial compounds during cold storage (Djenane *et al*. 2011; Aliakbarlu and Khalili Sadaghiani, 2015; Tajik *et al*. 2015) [10, 11, 12]. Among various types of natural products, essential oils (EOs) of aromatic and medicinal herbs have been widely accepted by consumers because of having both strong antimicrobial and antioxidant activities along with ephemeral and biodegradable nature (Hyldgaard *et al*. 2012; Prakash *et al*. 2015) [13. 14]. Amongst various essential oils which have been traditionally used Oregano occupies an important place. Oregano essential oil (OEO) is among the top 10 most popular essential oils (EO's) used as preservatives in food (Boskovic *et al*., 2019) [15]. It possesses strong antimicrobial, antifungal, and antioxidant activities (Bozin *et al*, 2006; Horosovˇa *et al*, 2006) [16, 17] along with antimutagenic and anticarcinogenic effects (Arcila-Lozano *et al.*, 2004) [18] and is categorized as GRAS (Generally Recognized as Safe (GRAS) by the Food and Drug Administration (Manso *et al*., 2014) [19] thus making it a suitable candidate for meat preservation.

Materials and Methods

Hind leg portions from the freshly dressed sheep carcasses (12-18 months old animal) were procured from the local market and subjected to deboning. The lean meat obtained was used for the preparation of the emulsion immediately. Animal fat used in the experiments was preferably obtained from the carcass of the same kill. Essential oils (USDA Food Grade), Chemicals, media, reagents and Packaging material (Food Grade) were obtained from Standard firms like Sigma Aldrich and Himedia India Pvt. Ltd.

Meat emulsion was prepared by utilizing meat mincer and bowl chopper for controlled, efficient and hygienic production, with the following formulations (g/1000g meat emulsion) as given in Table 1:

Table 1: Formulation (g/1000g) of different mutton emulsion treatments

Ingredients	Treatments			
	Control (C)	Positive Control (PC)	O100	O200
Mutton	800	800	800	800
Mutton Fat	200	200	200	200
Salt	25	25	25	25
Chilled Water	100	100	100	100
Butylated hydroxyl toluene (BHT)	0	0.2	0	0
Oregano essential oil (OEO)	0	0	0.1	0.2

The emulsion was divided into 4 treatments *viz*., Control (C)-without any additives, Positive Control (PC) containing synthetic additive BHT (Butylated hydroxyl toluene) @ 0.02% (200ppm), O100 containing Oregano essential oil (OEO) @ 0.01% (100ppm) and O200 containing Oregano essential oil (OEO) @ 0.02% (200ppm).

Samples from all the treatments were packaged aerobically in Low density polyethylene (LDPE) pouches (200µm gauge) and stored at refrigeration temperature (4±1ºC) for a period of 1 week during which they were analyzed at days 0, 3, 5, 7 for different parameters as follows:

Thiobarbituric acid reactive substances (TBARS)

TBARS was determined according to the method of Serrano *et al* (2006) [20]. 2 g sample from each batch in duplicate was homogenized in 8 ml of 5% trichloroacetic acid (TCA) with the help of Ultraturrax tissue homogenizer (T-25, Germany). The homogenized sample was filtered through ash less Whatman filter paper No. 1 and the filtrate was adjusted to 10 ml with 5% TCA. An aliquot of 5 ml of filtrate was mixed with 5 ml of 20 mM aqueous TBA and placed in dark for 20 h at room temperature. The absorbance was measured at a fixed wavelength of 532 nm using UV/VIS Spectrophotometer (Hitachi, UV-Spectrophotometer U-1800, Japan). A standard curve was plotted with 1,1,3,3- tetraethoxypropane to obtain the malonaldehyde (MDA) concentration and the results were expressed as mg MDA/kg of sample.

Peroxide Value

Peroxide value was determined by monitoring iodine liberated from Potassium Iodide (KI) by lipid peroxides. 2 gram of sample was dissolved in 20 mL of a mixture of glacial acetic acid: chloroform solution (3:2, v/v). 1 ml of saturated Potassium iodide solution was added to the sample and mixed vigorously. The mixture was allowed to stand for 5 minutes in dark at room temperature followed by addition of 30 mL distilled water. The mixture was gradually titrated with sodium thiosulphate solution (0.01 N) with vigorous shaking until the yellow color almost disappeared. Then 1 mL of 1% starch indicator was added and titration continued, adding the thiosulfate solution slowly until the blue color disappeared. Peroxide value (PV) was calculated and expressed in milliequivalents of active oxygen per kilogram (meq.O2/kg meat) using the following equation:

$$\text{PV (meq/Kg sample)} = \frac{\text{V x N}}{\text{W}} \text{ x } 1000$$

Where,
V= Volume of sodium thiosulphate used
N=Normality of sodium thiosulphate
W=weight of sample (g)

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity: Antioxidant activity of meat emulsion extracts based on DPPH (2,2 diphenyl-1-picryl hydrazyl) radical was analyzed following the method given by Brand Williams *et al.* (1995) [21]. The stock solution was prepared by dissolving 24 mg DPPH with 100ml methanol and then stored at -20 ^{0}C until needed. The working solution was obtained by mixing 10mL stock solution with 45ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using UV/VIS Spectrophotometer (Hitachi, UV-Spectrophotometer U-1800,

Japan). The sample extract was prepared by homogenizing 1 gram of sample with 25ml of methanol. The mixture was filtered through ash less filter paper No. 1. 150µl of the extract was allowed to react with 2850µl of the DPPH solution for 24 h in the dark. Sample blank was also prepared by reacting 150µl of the methanol with 2850µl of the DPPH solution. Then the absorbance was taken at 515 nm. The radical scavenging activity was measured using the formula:

Radical scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Total Plate Count (TPC)

TPC was determined according to standard procedure of APHA (2008) [22]. The cfu/g was calculated by taking average number of colonies and the bacterial counts were finally expressed as logarithms of colony forming units per gram of sample (log_{10}CFU/g).

Overall Acceptability

The Sensory evaluation of the emulsion was conducted as per Zhou *et al*. (2021) [23], wherein the samples were evaluated on the basis of five-point descriptive scale (5= Extremely desirable, 1= Extremely undesirable) to determine the Overall acceptability of emulsion samples.

Statistical analysis

The whole set of experiment was repeated thrice (3-trials) for consistency of the results and samples for each parameter were drawn in duplicate for analysis leading to total observation 6 (n=6); for sensory attributes seven panelists analysed the samples leading to total observations (n=21). The data generated from the experiments was pooled and subjected to statistical analysis using ANOVA and results were expressed as means with standard error. The means were compared using Duncan's multiple range test (DMRT), with a significance level of 0.05 using a SPSS package (SPSS 20.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Thiobarbituric acid reactive substances (TBARS) During storage an overall increase in TBA values was observed for control and treated samples (Table-2) wherein the TBA values increased significantly (p<0.05) on each storage day reaching a maximum value on day 7 of storage for control as well as treated samples due to progressive increase in lipid oxidation of the products. The use of Oregano essential oil and BHT helped in delaying lipid oxidation during 7 days of storage. Significant differences (p<0.05) in TBA values of all treatments were found on days 3 and 5 of storage. On day 7, the differences between PC, O100 and O200 were non-significant (p>0.05) to each other but significant (p<0.05) as compared to control. TBA values above 0.5 indicate some oxidation and values above 1 possibly unacceptable levels. (Warriss, P.D., 2000) [24]. The mean values of TBARS of all treatments except control during the storage period were way below the minimum threshold value, i.e., 1–2 mg malonaldehyde/kg meat (Greene & Cumuze, 1982; Watts, 1962) [25, 26]. The Control emulsion exceeded the threshold levels of 0.5 and 1 on day 5 and day 7 respectively. During the storage period, TBARS values increased as expected owing to the fact that lipid oxidation is an autocatalytic process and, therefore, the oxidation rate increases as the reaction proceeds (Smaoui *et al*., 2016) [27]. However, despite the general increase in TBARS, the values on day 7 of storage of treated samples were within the acceptable limit showing lower oxidations of the OEO and BHT treatments compared to CON, which may be related to phenolic compounds and antioxidant activity present in oregano essential oil (Kempinski *et al*., 2017) [28]. The antioxidant activity of phytochemicals in the essential oil is associated with the hydroxyl group attached to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals (Krishnan *et al*., 2104) and Falowo *et al*., 2019) [29, 30]. Phenolic and flavonoid compounds from essential oils prevent oxidation processes by donating electrons and interrupting propagation steps, sequestering free radicals, chelating metal ions, or acting as substrates for radicals such as superoxide or hydroxyl (Falowo *et al*., 2014 [31]; Viuda-Martos *et al*., 2009 [32]). Ünal etal. (2014) [33] reported that Oregano treatment displayed significantly lower TBA value than those of rosemary, sage and control treatments in minced beef during refrigerated storage. Botsoglou *et al*. (2003a) [34] and Botsoglou *et al*. (2003b) [35] in their studies on chicken and turkey meat respectively found that oregano essential oil protects meat against oxidation. Fasseas, *et al*. (2007) [36] reported that pork and beef added with 3% oregano essential oil showed lower levels of oxidation after 12 days of refrigerated storage.

Table 2: TBARS values (Mean ± SE) of different treatments of mutton emulsion during refrigerated storage (4±1ºC)

Treatment	Storage Period (Days)			
	Day-0	Day-3	Day-5	Day-7
C	$0.14\pm.01^{A}$	$0.41\pm.02^{B4}$	$0.59\pm.01^{C4}$	$1.38\pm.27^{D2}$
PC	$0.13\pm.003^{A}$	$0.18\pm.01^{B1}$	$0.28\pm.01^{C1}$	$0.36\pm.01^{D1}$
O100	$0.14\pm.01^{A}$	$0.37\pm.01^{B3}$	$0.47\pm.01^{C3}$	$0.50\pm.01^{D1}$
O200	$0.14\pm.003^{A}$	$0.24\pm.01^{B2}$	$0.34\pm.02^{C2}$	$0.44\pm.02^{D1}$

Mean ± S.E. with different superscripts row wise (A, B, C, D) and between column wise (1, 2, 3, 4) differ significantly (p<0.05).

Peroxide value (PV)

The peroxide value (PV) of meat and meat products during storage is a measure of the primary degree of oxidation. In general, the peroxide value is a helpful measure of the degree of oxidation of lipids, fats, and oils. Peroxide value determination has the benefit of directly measuring lipid peroxides, which are major lipid oxidation products. The PV (meq/kg sample) of all the treatments increased significantly (p<0.05) during storage from day-0 to day-5 (Table-3). However, the PV started to decrease as the storage period progressed from day 5 to day 7 which could be due to peroxide decomposition during the latter part of storage into secondary lipid oxidation products (Dominguez *et al*., 2019) [5]. Similar to this study, a decrease in the PV during storage was reported in the PV of chicken leg and breast meat (Soyer *et al*., 2010, Teets *et al*., 2008) [37, 38] during frozen storage which could be due to degradation into secondary oxidation products during the extended storage period. PC, O100 and O200 emulsions maintained significantly lower (p<0.05) PV as compared to C throughout the storage study except on day-0. The differences in PV of PC, O100 and O200 emulsions remained significant except on day-3 on which the PV of PC was comparable to O200. The lower peroxide value in treatment emulsions is an indication of antioxidant potential of Oregano essential oil (OEO), attributed to the phenolic

groups i.e. polyphenols (mainly flavonoids) which can retard peroxide formation in meat emulsion through hydrogen donation or radical scavenging resulting in impeding initiation and propagation stages of lipid oxidation. Similar observations were recorded in pork patties incorporated with sea buckthorn extract (Wagh *et al*., 2017) [39] and in pork emulsion containing tomato products and pink guava pulp (Joseph *et al*., 2014) [40] stored under aerobic packaging condition at 4±1 ºC respectively.

Table 3: Peroxide values (Mean ± SE) of different treatments of mutton emulsion during refrigerated storage (4±1 ºC)

Treatment	Storage Period (Days)			
	Day-0	Day-3	Day-5	Day-7
C	2.92±0.01 ^{A}	5.50±0.11 ^{B3}	7.30±0.03 ^{C4}	6.27±0.04 ^{D4}
PC	2.89±0.01 ^{A}	3.72±0.01 ^{B1}	4.83±0.02 ^{C1}	3.83±0.03 ^{D1}
O100	2.88±0.02 ^{A}	4.71±0.01 ^{B2}	6.27±0.04 ^{C3}	5.90±0.02 ^{D3}
O200	2.90±0.01 ^{A}	3.68±0.02 ^{B1}	5.64±0.16 ^{C2}	4.66±0.04 ^{D2}

Mean ± S.E. with different superscripts row-wise (A, B, C, D) and between column-wise (1, 2, 3, 4) differ significantly (p<0.05).

DPPH-RSA

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is based on the reaction where the purple-coloured DPPH (a stable free radical) is reduced to the yellow-coloured diphenylpicrylhydrazine when reacting with the free radicals of the sample (Kirby & Schmidt, 1997) [41]. The antioxidant activity of all samples decreased significantly (P<0.05) with storage time from day 0 to day 7 (Table-4). Control emulsion had significantly lower (p<0.05) DPPH activity than PC, O100 and O200 during the entire storage study. PC samples displayed significantly higher (p<0.05) antioxidant activity than both O100 and O200 samples throughout the storage except on day 0 when the values of PC and O200 were comparable. The results are in line with Boskovic *et al*. (2019) [15], who also reported that OEO had weaker antioxidant activity (p<0.05) than the synthetic antioxidant, BHT. Similar DPPH radical scavenging activity of OEO was reported by other authors (Aazza *et al*., 2011; Sokmen *et al*., 2004) [42, 43]. Ünal *et al*. (2014) [33] also reported that Oregano treatment had significantly (p<0.01) higher levels of antioxidant activity than rosemary, sage and control treatments in minced beef during refrigerated storage.

The antioxidant activity of OEO is directly related with their phenol content, especially that of oxygenated monoterpene thymol and carvacrol, which are considered to be mainly responsible for the antioxidant potential of OEO (Biskup *et al*, 2013) [44]. These compounds possess a hydroxyl group (–OH) that acts as an H atom donor to free radicals, thereby preventing oxidation of other compounds (Tongnuanchan & Benjakul, 2014) [45].

Table 4: DPPH-RSA values (Mean ± SE) of different treatments of mutton emulsion during refrigerated storage (4±1ºC)

Treatment	Storage Period (Days)			
	Day0	Day3	Day5	Day7
C	14.41±0.14 ^{D1}	11.60±0.28 ^{C1}	9.42±0.07 ^{B1}	7.11±0.07 ^{A1}
PC	34.19±1.53 ^{C3}	29.87±0.33 ^{B4}	27.15±0.61 ^{B4}	20.97±0.05 ^{A4}
O100	22.69±0.25 ^{D2}	20.65±0.37 ^{C2}	16.23±0.37 ^{B2}	12.40±0.20 ^{A2}
O200	33.91±1.12 ^{D3}	27.25±0.51 ^{C3}	24.70±0.31 ^{B3}	18.29±0.17 ^{A3}

Mean ± S.E. with different superscripts row-wise (A, B, C, D) and between column-wise (1, 2, 3, 4) differ significantly (p<0.05).

Total Plate Count (TPC)

TPC values for all treatments are given in Table-5. The initial value of TPC (day 0) for all the treatments was around 3 log cfu/g, indicative of good quality emulsion. TPC did not reach a value of 7 log cfu/g, considered as the upper microbiological limit for good quality fresh meat as defined by the ICMSF (1986) [46]. There was a significant (p<0.05) increase in the TPC in all Day-7 samples as compared to Day-0 treatments. Both O100 and O200 had significantly lower (p<0.05) counts than C and PC on each storage day indicative of the antimicrobial effect of Oregano essential oil. The results are in agreement with Chouliara *et al*. (2007) [47] who reported reduced TVC (Total Viable Count) in fresh chicken breast meat by the combination of MAP and oregano essential oil. Tsigarida *et al*. (2000) [48] also reported a reduction in initial microflora of beef meat fillets by 2–3 log cfu/g with the addition of 0.8% of oregano essential oil. Similarly, Scandamis and Nychas (2001) [49] reported an immediate suppression of TVC in minced beef meat by 1 log cfu/g when OEO was added at concentration of 1%. Zhang *et al*. (2010) [50] also reported that OEO could extend the shelf-life of fresh chicken breast meat by reducing the growth of microorganisms during refrigerated storage. OEO (0.02%) treatment in conjunction with orange dietary fibre (1%) and vacuum packaging conditions increased the shelf-life of bologna sausages (Viuda-Martos *et al*., 2010) [51]. El Adab and Hassouna (2016) [52] found that the addition of OEO significantly (p<0.05) reduced the TVC in poultry meat sausage. Dzudie *et al*. (2004) [53] reported that the addition of EOs significantly reduced (p<0.05) the number of TVC in beef patties. The antibacterial activity of EOs is not dependent on a single mechanism, and the action varies depending on the components of the microorganism (Pateiro *et al*. 2021) [54]. Mechanisms for the activities of chemical components in EOs have been hypothesised (Burt 2004) [55]. Membrane disruption is the most common mechanism of antibacterial action (Pateiro *et al*. 2021) [54]. Bioactive compound accumulation in the cytoplasmic membrane's phospholipid bilayer causes cytoplasmic membrane damage, increased fluidity and permeability, leakage of intracellular constituents, disruption of embedded proteins, and cell death (Calo *et al*. 2015 [56]; Huang *et al*. 2014; Pateiro *et al*. 2021) [57, 54]. The main antibacterial chemicals discovered in OEO are carvacrol and thymol. Thymol and carvacrol can disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharide components and increasing the permeability of adenosine triphosphate in the cytoplasmic membrane, thereby changing the cell's passive permeability (Guarda *et al*., 2011) [58].

Table 5: Total Plate Count (log_{10}CFU/g) of different treatments of mutton emulsion during refrigerated storage (4±1 ºC)

Treatment	Storage Period (Days)			
	Day 0	Day 3	Day 5	Day 7
C	3.30±1.06^{A}	4.78±0.29^{AB2}	6.02±0.12^{BC2}	6.74±0.13^{C2}
PC	3.36±0.37^{A}	4.69±0.29^{B2}	6.11±0.32^{C2}	6.68±0.09^{C2}
O100	3.16±0.03^{A}	4.14±0.12^{B12}	5.04±0.0^{C1}	6.39±0.28^{D12}
O200	3.02±1.06^{A}	3.55±0.21^{A1}	4.85±0.23^{AB1}	5.90±0.03^{B1}

Mean ± S.E. with different superscripts row-wise (A, B, C, D) and between column-wise (1, 2, 3, 4) differ significantly (p<0.05).

Overall Acceptability

Overall acceptability of the product was based on parameters like colour, odour and texture of the emulsion samples. During storage an overall decrease in the mean overall acceptability scores (Table-6) were observed for C and PC samples but for O100 and O200 samples the acceptability scores increased from days 0 to 3 and then decreased from days 3 to 7. On day 0, C and PC had significantly higher scores than O100 and O200, which may be because of the residual aroma of the essential oils imparted to the product leading to lowering the overall acceptability scores of O100 and O200 during the initial days of storage. However as the storage progressed and oxidation was induced the overall acceptability scores of C started diminishing and was significantly less (p<0.05) than the treated emulsions on days 5 and 7. At the end of storage period PC and O200 had significantly higher (p<0.05) overall acceptability scores than C and O100, while as O100 had significantly higher scores than C. The Overall acceptability scores coincide with TBARS and TPC scores showing the direct relationship of oxidative and microbial evolution with the acceptability of the product and hence establishing the anti-oxidative and antimicrobial potency of OEO which according to this study was dose dependent with O200 displaying better protective effect than O100. Chouliara *et al*. (2007) [47] also reported a decreasing trend in the acceptability scores of fresh chicken breast meat stored at refrigeration temperature as the storage progressed. On the basis of sensory evaluation Chouliara *et al* (2007) [47] reported a shelf-life extension of breast chicken meat by 3–4 days for samples containing 0.1% oregano oil and found that the OEO at 1% gave a very strong unacceptable odour and taste to the product as compared to 0.1% concentration. Our results also agree with Karabagias *et al*. (2011) [59] who reported the lower acceptability score of 2 was reached for odour after 7 days for air packaged samples. Govaris *et al*. (2010) [60] reported that 0.6% OEO displayed better sensory scores than 0.9% in minced sheep meat. Our results also coincide with Al-Hijazeen (2018) [61] who found that oregano odour was very clear and highly significant (p<0.05) in all samples of chicken patties added with OEO compared to the other treatments. However, the oxidation odor in the latter parts of storage were lowest for OEO treated sample with lowest overall acceptability for control samples and the best values for OEO and sodium nitrite treated samples. These results also agreed with Al-Hijazeen *et al*. (2016) [62] who studied the effect of adding different level of oregano oil (Origanum vulgare) on sensory attributes of both raw and cooked chicken meat. Adding essential oils showed positive effect on the overall meat quality and visual properties such as color and aroma (Kahraman *et al*., 2015; Vital *et al*., 2016) [63, 64].

Table 6: Overall acceptability scores (Mean ± SE) of different treatments of mutton emulsion during refrigerated storage (4±1 °C)

Treatment	Storage Period (Days)			
	Day 0	Day 3	Day 5	Day 7
C	5.00±0.00 ^{D2}	4.33±0.21 ^{C12}	3.00±0.26 ^{B1}	2.17±0.17 ^{A1}
PC	5.00±0.00 ^{C2}	4.67±0.21 ^{BC2}	4.17±0.40 ^{AB2}	3.67±0.21 ^{A3}
O100	3.00±0.26 ^{A1}	4.17±0.17 ^{B12}	3.33±0.21 ^{A1}	2.84±0.17 ^{A2}
O200	2.83±0.17 ^{A1}	3.83±0.31 ^{BC1}	4.33±0.21 ^{C2}	3.50±0.22 ^{AB3}

Mean ± S.E. with different superscripts row-wise (A, B, C, D) and between column-wise (1, 2, 3, 4) differ significantly (p<0.05).

Conclusion

Meat and meat products are high in nutrients, which promote oxidation as well as the growth of spoilage and harmful microorganisms. As clean-label alternatives, essential oils (EO's) can avoid the carcinogenic and hazardous issues that synthetic food additives produce. The biological activity of EO's is inextricably linked to their bioactive constituents, particularly phenolic compounds. In the present study, based primarily on overall acceptability scores of ≥3 as satisfactory scores, the shelf-life of aerobically packaged control meat emulsion was 5 days maximum. Addition of 0.01% oregano essential (O100) although enhanced the acceptability scores but the scores were still less than 3 at the end of storage. Both BHT (PC) and 0.02% oregano essential oil (O200) extended product shelf life by 2-3 days with acceptability scores of >3 at the end of storage. The oregano essential oil did increase the shelf life of meat emulsion, thus offering a potential option for the food industry to replace synthetic additives by natural ones to preserve meat quality and retain consumer acceptance. In conclusion, oregano essential oil may extend the shelf life of meat products. However, the study also suggests that for food models, a larger concentration of EO's is needed, thus limiting their addition in meat because of the possible adverse effect on sensory properties of the product. The most common counterintuitive and challenging difficulty with using EO's in food products is keeping organoleptic features of food items with relatively modest EO concentrations while retaining effective antioxidative and antimicrobial abilities. Encapsulating EO's in one or more wall materials that controllably carry, deliver, and release EO's is one of the novel solutions to this problem which can be further enhanced by opting for micro or nano-encapsulation of essential oils for inclusion in meat products thereby enhancing their overall activity as well as countering their adverse sensory effects.

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